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LEVELS OF METHYLMERCURY AND CONTROLLING FACTORS IN SURFACE SEDIMENTS OF THE CARSON RIVER SYSTEM, NEVADA

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Abstract

Spatial and temporal distribution of methylmercury (MeHg) was determined in surficial sediments collected from a river–reservoir system impacted by Hg-contaminated mine wastes. Despite the fact that total mercury concentrations (Hg_T) in surface sediments of the Carson River system were in the $\mu g.g^{-1}$ range, levels of MeHg varied from about 2 to 28 $ngHg.g^{-1}$ dry weight, representing less than 3% of Hg_T. Concentrations of MeHg were well correlated with both the biotic ($r = 0.95$) and abiotic activity ($r = 0.85$) of the sediment, determined as the ability of each compartment to specifically reduce an alternative electron acceptor. However, the positive relationship between the two measured activities suggests that the abiotic activity may be due to reductant substances produced by micro-organisms. When sediments collected from the Carson River were used in laboratory assays for the determination of potential rates of MeHg production, the addition of inorganic Hg (added as $HgCl_2$) resulted in increased rates of methylation when the spike concentration was lower or equal to 15.3 $\mu g.g^{-1}$ dry weight. This trend was reversed for spike concentration of inorganic Hg above 15.3 $\mu g.g^{-1}$. The reduction of methylation rate was associated with an inhibition of microbial activity. These observations suggest that seasonal inputs into the river of significant amounts of inorganic Hg eroded from mill tailings during winter and spring flooding events could have an inhibiting effect on Hg-methylating micro-organisms. This observation could explain the low $[MeHg]/[Hg_T]$ ratios previously documented in waters of the Carson River system. Copyright © 1996 *Published by Elsevier Science Ltd*

Keywords: Mining activity, sediment, Hg, microbial activity, methyl-Hg production.

INTRODUCTION

Elevated levels of mercury (Hg) found in fish tissues have generated public concern over human health risks associated with consumption of contaminated fish (Bjorkland *et al.*, 1984; Fitzgerald & Clarkson, 1991; Lindqvist, 1991). The ecological and human health effects of Hg are generally related to the environmental transformation of inorganic Hg to the toxic and biomagnification-prone methyl mercury (MeHg) (Compeau & Bartha, 1985). More than 80% of Hg accumulated in fish tissues is present as MeHg (Grieb *et al.*, 1990; Bloom, 1992). However, processes leading to MeHg production in natural aquatic systems are still poorly understood. Despite some disagreements over whether the methylation and demethylation of Hg^{2+} are biologically or abiotically mediated, experimental evidence is available to support contributions from both mechanisms in natural environments (Weber, 1993). Previous investigations on the methylation of Hg have shown that MeHg production occurs in sediment (Compeau & Bartha, 1985; Xun *et al.*, 1987; Gilmour & Henry, 1991; Gilmour *et al.*, 1992), as well as in the water column (Xun *et al.*, 1987). Even though the degree to which sediments act as a source of MeHg to aquatic biota is unknown, both inorganic Hg and MeHg are mainly concentrated in sediments relative to the water column (Gilmour & Henry, 1991).

The Carson River system in Nevada has been contaminated by mine wastes for over a century by the historic Comstock mines. Precious metals were extracted by an amalgamation process in which liquid Hg was used to free the gold and silver from milled ores. A significant portion of Hg-contaminated waste tailings generated by this activity have directly or indirectly entered the Carson River (Smith, 1943) and accumulated in bed and river bank sediments (Cooper *et al.*, 1985). Several investigations have been conducted recently to document the extent of Hg contamination in the Carson River drainage basin (Cooper *et al.*, 1985; Gustin *et al.*, 1994); however, no data exist on concentrations of MeHg in sediments.

The purpose of our study was to determine concentrations of MeHg in surficial sediments along the Carson River, as well as factors controlling MeHg production in sediment.

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MATERIALS AND METHODS

Study area

The area under investigation is a 100 km section of the Carson River drainage basin in western Nevada, which has been described in detail elsewhere (Fig. 1) (Cooper *et al.*, 1985; Gustin *et al.*, 1994; Bonzongo *et al.*, 1994). Six sampling stations were selected based on the distribution of Hg along the system (Cooper *et al.*, 1985). Deer Run Road (station 1, Fig. 1) is located upstream of the contaminated mine tailings. The introduction of Hg into the river from contaminated mine tailings occurs primarily at Dayton (station 2, Fig. 1). The remaining sampling stations were located downstream from this site and within the contaminated section of the river–reservoir system. Surficial sediment samples (0–5 cm) were collected in June 1994, January and July 1995. At each station, three sediment samples (at approximately 1 kg) were collected in Whirl-pack bags by manual scooping using polyethylene shovels. During the sampling period and transportation, samples were stored in a cooler containing ice packs. In the laboratory, samples were transferred to the refrigerator and stored at 4°C before analyses.

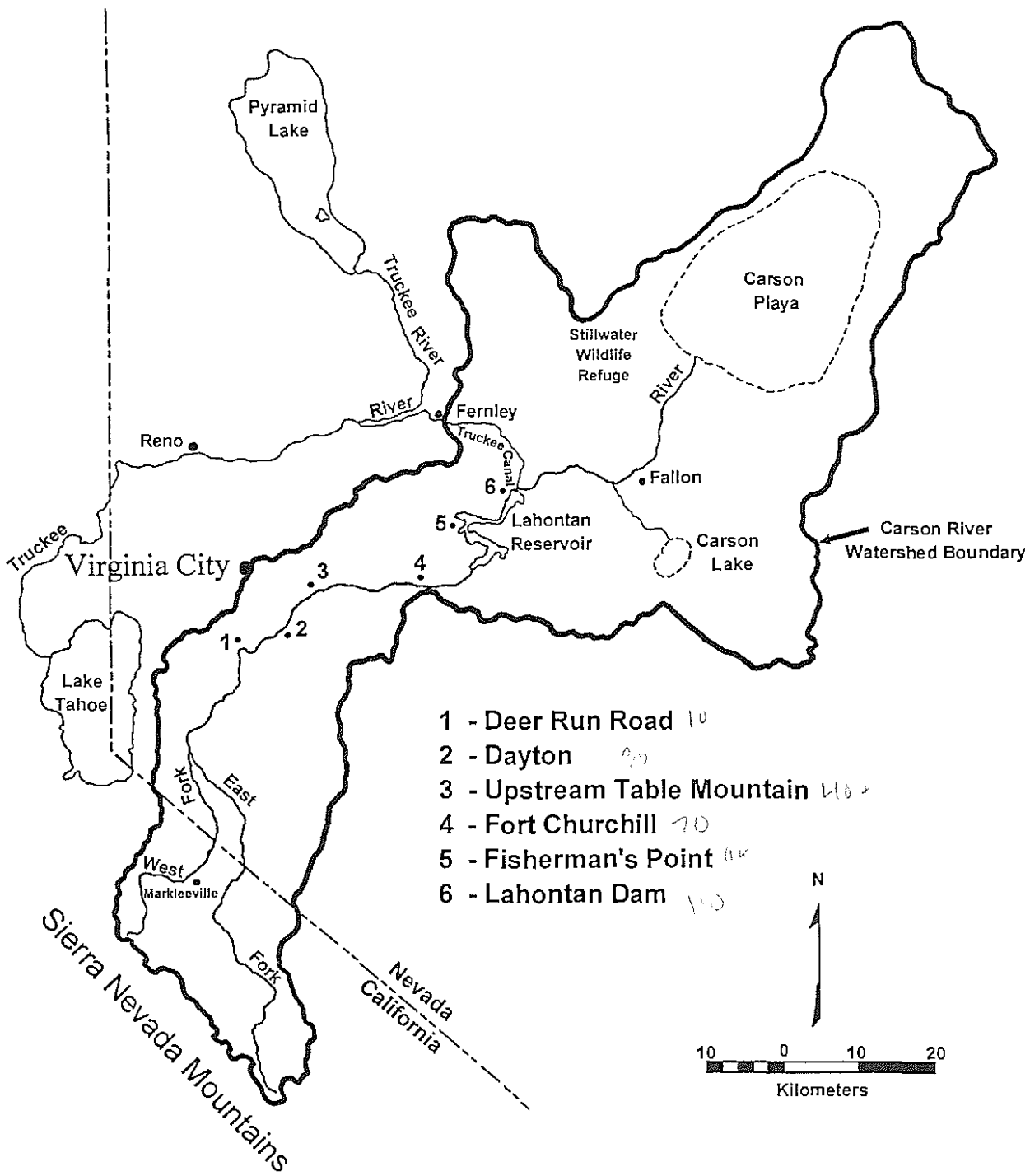

Fig. 1. Location of sampling sites along the Carson River system.

Total mercury and methylmercury analyses

The sample analyzed at each point was a composite of three separate samples. In June of 1994, Hg_T was determined on approximately 10 g of wet sediment homogenate. The sample was digested with 10 ml of HNO_3/HCl (1:3, v/v) under gentle reflux conditions for 2 h. The analysis was performed by cold vapor atomic absorption (CV–AA). The detection limit was 10 ng/g. In January and July of 1995, Hg_T was determined on 0.5–1 g of wet sediment homogenate digested with 10 ml of HNO_3/H_2SO_4 (7:3, v/v) under gentle reflux conditions for 8 h. The detection limit was ~1 ng/g. In both cases, method development and quality assurance were tied to US NIST Hg standard reference materials. MeHg determination was performed using a method adapted from Horvat *et al.* (1990), and each sample was analyzed in triplicate. Briefly, MeHg was extracted from fresh sediment samples by a mixture of 2 M H_2SO_4, (saturated with $CuSO_4$) and a 4 M solution of KBr. MeHg was then extracted in toluene. A cysteine cleanup step was then undertaken to remove constituents which can potentially interfere with chromatographic resolution of MeHg. MeHg was determined using a Hewlett-Packard 5890 gas chromatograph with an electron capture detection (GC–ECD), and a capillary column packed with 80% FFAP, obtained from Supelco. The detection limit was ~2 ng/g. The precision of the method based on the variation coefficient of six analyses of the sample collected from Table Mountain (station 3, Fig. 1) in June of 1994 was 6.7% (29.3 ± 1.9, average ± one standard deviation). The percent recovery of MeHg from spiked sediment samples and standard reference material (DORM-2, National Research Council Canada, certified value: 4.47 ± 0.32 μg MeHg as $Hg.g^{-1}$) averaged 93 ± 3% ($n = 5$) and 97 ± 5% ($n = 5$) respectively.

Biotic and abiotic activity of sediment samples in relation to methylmercury production and accumulation

Much of the Hg found in aquatic systems is accumulated in sediments. Its mobility and ultimate fate are controlled by a variety of biotic and abiotic factors (Gilmour & Henry, 1991). In both marine and freshwater sediments, the biotransformation of ionic Hg has been attributed to microorganisms, primarily sulfate reducing bacteria. Evidence for this involvement is observed when molybdate (MoO_4^{2-}), a sulfate reduction inhibitor, is added to sediment samples and rates of Hg methylation are significantly reduced (Compeau & Bartha, 1985; Gilmour *et al.*, 1992). Abiotic methylation is also observed (Weber, 1993), but is poorly understood, and the relative contribution of biotic and abiotic processes to the pool of MeHg produced and/or accumulated in aquatic environments is unknown (Weber, 1993; Gilmour & Henry, 1991). The latter observation is attributable to the lack of analytical procedures able to discriminate between biological and nonbiological methylation of Hg without changing both the chemistry and biology of the sample.

In this study, biotic and abiotic activity of sediment samples, defined as the potential of each compartment to selectively reduce an alternative electron acceptor (Liu & Strachan, 1981; Trevors, 1984), were used to assess the relative influence of both biological and nonbiological processes on levels of MeHg produced and/or accumulated in sediments. For this purpose, two methods were used.

The first method, described by Liu and Strachan (1981), is suitable for a direct determination of abiotic activity. The method is a spectrophotometric test based on the quantitative reduction of the dye resazurin by both chemically reducing substances and dehydrogenase in microorganisms. The abiotic and biotic activities are differentiated by using *m*-cresol to inhibit microbial activity. The activity is expressed as μg of resazurin reduced per day and per gram of sediment.

The second method is based on the measurement of the electron transport system (ETS) activity of respiring microbes, giving a direct measurement of biotic activity in sediment samples (Trevors, 1984). In this method, an electron acceptor, the 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT), is reduced to iodonitrotetrazolium (INT–formazan). The INT–formazan produced is extracted in methanol and its absorbance is determined spectrophotometrically at 480 nm.

Hg(II) levels and methylation potentials in surface sediments

Sediment from Table Mountain (station 3, Fig. 1) was used in laboratory assays to estimate the effect of different Hg concentrations on the production of MeHg by microorganisms, naturally adapted to high levels of Hg. Sediments from this location were chosen because of the natural occurrence of the highest concentration of both Hg_T (14 100 $ng.g^{-1}$) and MeHg (28.5 ng $Hg.g^{-1}$). Homogenized sediment samples were slurried in acid cleaned BOD bottles with double distilled water and the pH was adjusted to 7.4 with 1 N NaOH. Slurries were prepared in 1/10 ratios (equivalent of 1 g dry weight (dw) in 10 ml of double distilled water). Initial calculated concentrations in slurries without added Hg were approximately 14.1 $\mu g.g^{-1}$ for Hg_T and 0.028 $\mu g.g^{-1}$ for MeHg. Slurries were then spiked with Hg, added as $HgCl_2$. Spike concentrations of inorganic Hg tested were: 0 (no addition), 1.1, 1.52, 3.05, 15.3, 30.5, 91.4 and 152 $\mu g.g^{-1}$ dw. Each concentration was tested in triplicate and for each experimental batch, an acid killed control was conducted. After an incubation period of 48 h, both MeHg and microbial activity were determined in duplicates.

Total organic matter (TOM)

Total organic matter (TOM) in sediment samples was measured as loss on ignition by ashing at 550°C for 2 h. TOM (%) for the respective sampling points was determined to be 1 (4.5%), 2 (2.8%), 3 (3.4%), 4 (2.1%), 5 (0.7%) and 6 (3.8%).

RESULTS AND DISCUSSION

Superficial sediments collected from the Carson River system during the three sampling events contained low organic content (2–5%). Concentrations of Hg_T showed two ranges of values (Fig. 2a). From Deer Run Road downstream to Dayton (background region, Fig. 1), concentrations of Hg_T averaged 1500 $ng.g^{-1}$, which is similar to the range of values given for many uncontaminated river and lake sediments (Forstner & Wittman, 1983; Gilmour & Henry, 1991). Downstream of Dayton, Hg_T concentrations were one order of magnitude higher than those obtained from the background region, indicating the influence of Hg-contaminated mill tailings. Hg introduced into the river from mill tailings was found to be mainly bound to particles (Cooper *et al.*, 1985; Bonzongo *et al.*, 1994; Wayne *et al.*, in press), and its significant removal from the water column occurred in conjunction with the sedimentation process that takes place prior to reaching the dam site (station 6, Fig. 1) (Cooper *et al.*, 1985; Bonzongo *et al.*, 1994). This removal of Hg-contaminated particles from water could explain the decrease of Hg_T concentration in Lahontan Reservoir (locations 5 and 6, Fig. 1). Overall, the distribution of Hg_T in this study was similar to trends of Hg_T previously observed in both surface waters and sediments of the Carson River system (Cooper *et al.*, 1985; Bonzongo *et al.*, 1994; Wayne *et al.*, in press).

Mean concentrations of MeHg varied from less than 2 to 28.5 $ng\,Hg.g^{-1}$ (Fig. 2b). Overall, MeHg represented less than 3% of Hg_T concentrations, but its absolute values were one order of magnitude higher than ranges of MeHg concentrations determined on uncontaminated sediments (Ramlal *et al.*, 1987; Gilmour & Henry, 1991; Gilmour *et al.*, 1992). There is relatively little information on levels of MeHg in Hg-contaminated freshwater sediments for comparison. In Berry's Creek sediments (New Jersey, USA), severely polluted by mercury, Hg_T and MeHg concentrations ranged from 9 to 450 $\mu g.g^{-1}$ and from <1 to 8 $ng\,Hg.g^{-1}$ respectively (Berman & Bartha, 1986). Despite the high levels of Hg_T in Berry's creek sediments, Hg methylation activity was found to be limited by the unusually high concentrations of sulfide in sediments rather than by factors as pH, E_h, and Hg-methylating microbial activity (Berman & Bartha, 1986). In the Carson River–Lahontan Reservoir system, MeHg production in sediments also could be affected by high levels of sulfide in the anaerobic sediments of the reservoir (Cooper *et al.*, 1983; Miller *et al.*, 1994).

In this study, we have found that in the Carson River system, the accumulation and/or production of MeHg in June of 1994, were related to both the abiotic and biotic activity of sediments (Fig. 3). The temperature at the sediment–water interface during that period varied from 20 to 25°C. In both January and July of 1995, the temperature of water was lower (~10°C in January and ~17°C in July), and similar relationships were observed between MeHg concentrations and both biotic ($r=0.84$) and abiotic ($r=0.97$) activity. However, in contrast to

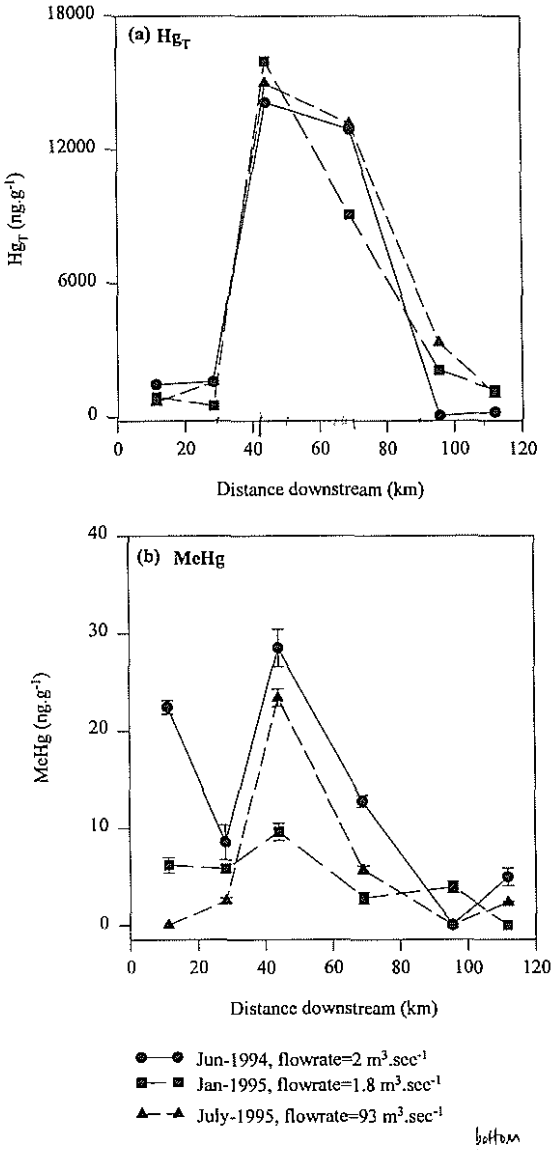

Fig. 2. Distribution of Hg_T (a) and MeHg (b) in sediment of the Carson River system. Samples were collected from each station in June 1994, and January and July 1995.

June 1994, where high biotic and abiotic activity were observed at location 1 and 3, only the latter exhibited high biotic and abiotic activity in 1995. These observations suggest that differences observed in concentrations of MeHg at each location during the three sampling events (Fig. 2) are attributable to several factors including temperature and both biotic and abiotic activity in sediment. Overall, the biotic activity was positively correlated to abiotic activity ($r=0.94$), suggesting that the abiotic activity was probably linked to reductant substances produced by micro-organisms.

When concentrations of Hg measured at the dam site were excluded because of its dissimilarity, we obtained a strong and positive relationship ($r=0.98$, Fig. 4) between percentages of MeHg determined in surface sediment in this study and those in water samples (Bonzongo *et al.*, 1994). This relationship suggests that the water column could be fed by both a vertical transfer of dissolved Hg from bed sediments, and resuspension of Hg-contaminated particles.

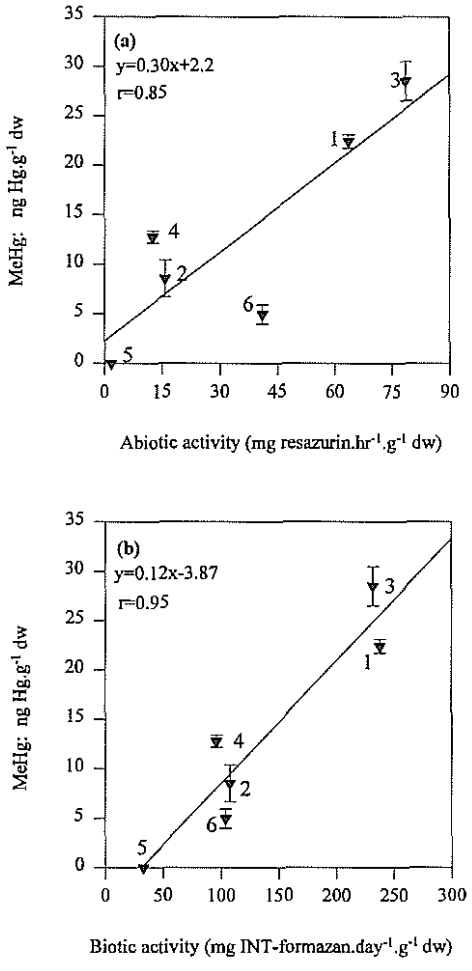

Fig. 3. Relationships between MeHg accumulated in surficial sediments and: (a) the abiotic activity; (b) the biotic activity of sediments. Results are expressed on a dry weight (dw) basis. Vertical bars are standard deviation. Numbers indicate sampling sites (see Fig. 1).

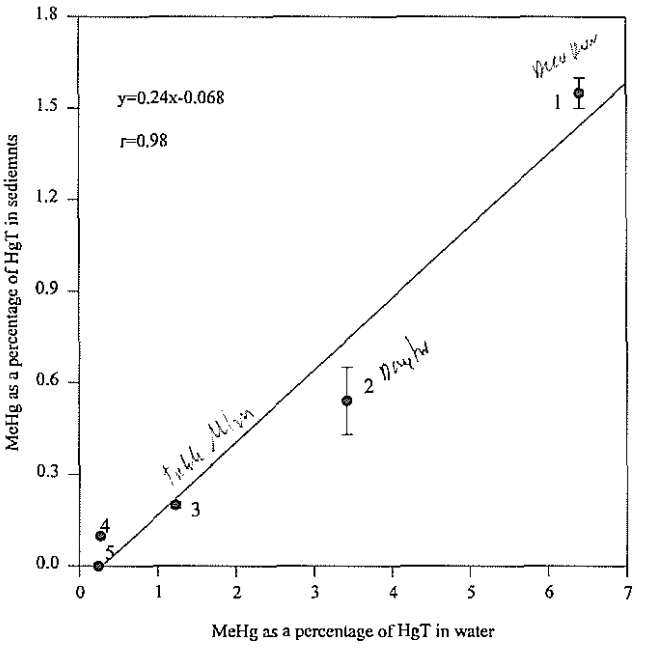

Fig. 4. Correlation between MeHg expressed as percentages of Hg_T determined on water and that on surface sediment samples collected from the Carson River system. Location 6 is excluded (see text for details). Numbers indicate sampling sites (see Fig. 1).

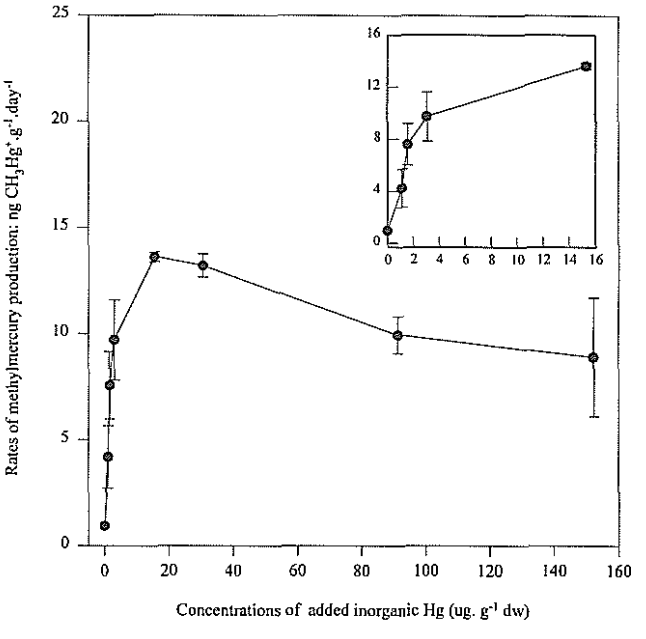

Fig. 5. Correlation between the MeHg production and the concentration of added inorganic Hg. Vertical bars are standard deviation. Inset is the same experiment over a narrower concentration range of added inorganic Hg.

In the Carson River drainage, mercury has been distributed in sediments downstream from the precious metal mill and associated with tailings disposal sites. Accordingly, inorganic mercury is resuspended during winter and spring flooding events and provides a periodic source of inorganic mercury to the Carson River system. We have assessed the impact of periodic inputs of inorganic Hg on the potential of MeHg production and microbial activity in sediments. For that purpose, experimental studies were conducted using sediment slurries spiked with increasing inorganic Hg concentrations, added as $HgCl_2$ (Fig. 5, Table 1). The addition of inorganic Hg in concentrations less than or equal to 15.3 $\mu g.g^{-1}$ dw result in an increase of methylation rate (Fig. 5). Correspondingly, inorganic Hg added in this range had low inhibitory effect on biotic activity. The electron transport system (ETS) activity of respiring micro-organisms in spiked samples was 4–17% lower than the control (Table 1). However, an opposite trend of methylation rate was observed when concentration of added Hg were higher than 15.3 $\mu g.g^{-1}$ dw, and percentage inhibition of ETS activity range from 25 to 42%. These results suggest that high concentrations of inorganic Hg depress MeHg production and/or accumulation. The more likely explanation for this observation is linked to microbial activity, which can be affected by high concentrations of Hg(II) (Fox & Walsh, 1982; Trevors, 1983; Robinson & Tuovinen, 1984; Barkay, 1987). Since the Carson River system has been heavily contaminated by Hg for over a century, microbial communities have had time to respond to Hg stress.

Table 1. Effect of $HgCl_2$ on ETS activity in sediment incubated aerobically

Concentration of $HgCl_2$[a] ($\mu g\ Hg^{2+}.g^{-1}$)	ETS activity[b] (μg INT-formazan.$day^{-1}.g^{-1}$ dw)	Percent inhibition
Control (0)	112.31 ± 14.8	Control (0)
1.11	107.52	4.26
3.05	102.60 ± 9.8	8.65
15.32	93.90 ± 16.0	16.39
30.47	84.56 ± 15.3	24.71
91.41	69.43 ± 5.7	38.18
152.34	65.27 ± 4.4	41.88

[a]Concentration of added inorganic Hg.
[b]Mean ± standard deviation.

It is known from laboratory investigations that microbial communities adapt to Hg stress through metabolic and genetic changes, which result in new physiological capabilities, allowing the biotransformation of Hg as a detoxification mechanism (Robinson & Tuovinen, 1984; Barkay, 1987). Both inorganic and organic forms of Hg are reduced to Hg^0 by specific enzymes (mercuric reductase and organomercury lyase), with subsequent volatilization (Fox & Walsh, 1982; Robinson & Tuovinen, 1984; Barkay, 1987). Hence, the availability of Hg(II) for methylation, as well as the accumulation of MeHg produced in these conditions are affected by the activity of these specific enzymes. From the results obtained in this study, we speculate that the production of MeHg in surface sediments of the Carson River could be reduced by the introduction of inorganic Hg from mill tailings into the river, introduction which occurs annually in conjunction with winter and spring runoffs (Cooper *et al.*, 1985; Bonzongo *et al.*, 1994). This aspect of the cycling of Hg could also explain, at least partly, the low percentages of MeHg in surface sediments of the Carson River, in comparison with sediments with lower Hg_T levels (Gilmour & Henry, 1991; Gilmour *et al.*, 1992).

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